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Investigating on *Besnoitia besnoiti* (Apicomplexa, Sarcocystidae) in naturally infected dairy cattle by an integrated approach.

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Bovine besnoitiosis, caused by *Besnoitia besnoiti*, is a (re)emerging disease in Europe (Cortes et al., 2014), including Italy (Gazzonis et al., 2014; 2017). However, its economic impact is scarcely considered and generally underestimated and there are still little studied aspects concerning both the parasite and the disease. Following a natural outbreak of besnoitiosis in a dairy herd, a study was planned to characterize *B. besnoiti* infection in cattle through a multidisciplinary approach.

Suspicious abortions and clinical cases of besnoitiosis were reported in a dairy farm (September 2017, Northern Italy) housing 216 Holstein cattle. Blood samples were collected; haematological and serological analyses (ELISA and confirmatory WB) were performed (Fernandez-Garcia et al., 2009). Histology and molecular (endpoint ITS-1 PCR (Cortes et al., 2007) and sequencing) analyses of tissues from a slaughtered cow with chronic besnoitiosis were carried out.

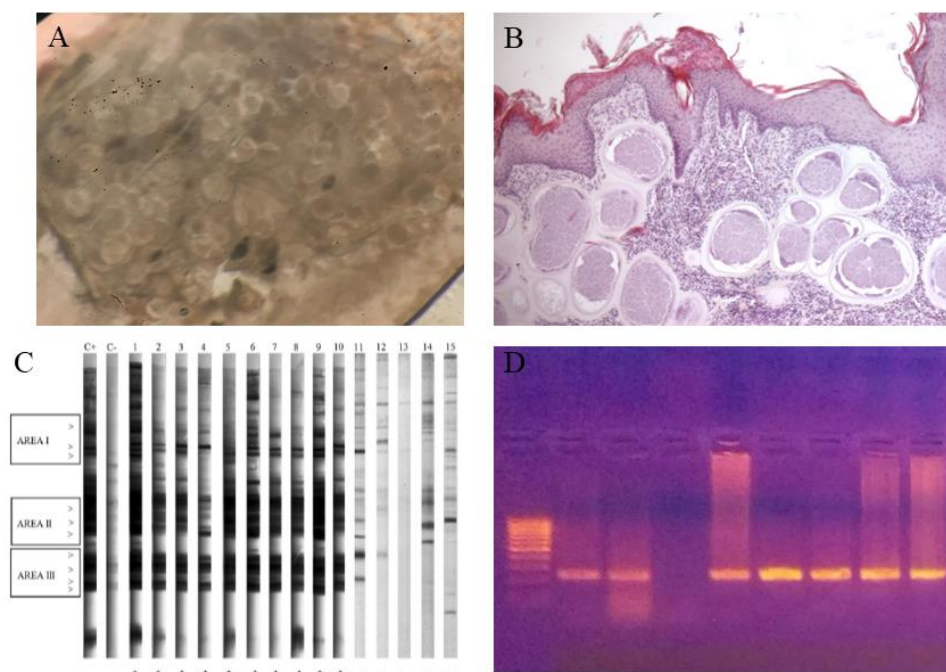
Out of 59 ELISA-positive animals, 50 (23%) were confirmed by WB. *B. besnoiti* prevalence was higher in cows (41%) than in calves (12%); no heifer resulted positive. Considering haematological parameters, a significant shift in the differential leucocyte formula from lymphocyte (L%) to granulocyte (G%) was recorded in infected cows (Mean±S.D.: L=46.1±18.4, G=53.9±18.4) if compared to negative animals (Student's T-test, p=0.012). Histology revealed a high load of *B. besnoiti* tissue cysts in skin, vulva, muzzle, sclera, eyelid, respiratory tract, emphasizing the possibility of parasite transmission through direct contact among animals. *B. besnoiti* was confirmed by PCR in other organs (heart, liver, aorta wall, tonsil) and especially in ovary, uterus and vulva, suggesting that the infection could affect cows' fertility. Parasite DNA was also found in masseters posing an important question for food security, even if *B. besnoiti* is not considered zoonotic (Figure 1). The study suggests that to investigate the dynamics of bovine besnoitiosis is mandatory to associate clinical and laboratory tests, including the genetic characterization of the parasite and its eventual correlation with the disease outcome.

Ethical statement: all procedures for collection of biological specimens from live animals were accomplished following good clinical practices in the respect of animal welfare according to

current legislation. The study was conducted with the approval of Institutional Animal Care and Use Committee of Università degli Studi di Milano (Permission OPBA_34_2017).

Conflict of interest statement: the authors declare they have no conflict of interest.

Figure 1: (A) Typical skin lesions in the chronic phase of besnoitiosis in a dairy cow. (B) Histological section of skin: hyperkeratosis with lymphohistiocytic and eosinophilic flogosis. (C) Pattern of recognition of *Besnoitia besnoiti* tachyzoite antigens by sera from naturally infected cattle by Western Blot. (D) Agarose gel-electrophoretic analysis of amplification products from conventional *Besnoitia besnoiti* ITS1 rDNA PCR on tissues from a slaughtered cow with chronic besnoitiosis



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